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| (54) Title: MICROPORATION OF HUMAN SKIN FOR DRUG DELIVERY AND MONITORING APPLICATIONS | | |
| (57) Abstract <p>A method of enhancing the permeability of the skin (120, 274) to an analytic for diagnostic purposes or to a drug for therapeutic purposes is described utilizing micro-pore and optionally sonic energy and a chemical enhancer. If selected, the sonic energy may be modulated by means of frequency modulation, amplitude modulation, phase modulation, and/or combinations thereof. Micro-pore is accomplished by (a) ablating the stratum comeum (274) by localized rapid heating of water such that water is vaporized, thus eroding cells; (b) puncturing the stratum comeum (274) which a micro-lancet calibrated to form a micro-pore of up to about 1000 μm in diameter; (c) ablating the stratum comeum (274) by focusing a tightly focused beam of sonic energy onto the stratum comeum (274); (d) hydraulically puncturing the stratum comeum (274) with a high-pressure jet of fluid to form a micro-pore of up to about 1000 μm in diameter; or (e) puncturing the stratum comeum (274) with short pulses of electricity to form a micro-pore of up to about 1000 μm in diameter.</p> <div data-bbox="860 1165 1453 1659" data-label="Image"> </div> | | |

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MICROPORATION OF HUMAN SKIN FOR DRUG DELIVERY
AND MONITORING APPLICATIONS
CROSS-REFERENCE TO RELATED APPLICATIONS

10

This application is a continuation-in-part of application Serial No. 08/520,547, filed August 29, 1995, which is a continuation-in-part of Serial No. 08/152,442, filed November 15, 1993, now U.S. Patent No. 5,458,140, and of Serial No. 08/152,174, filed December 8, 1993, now U.S. Patent No. 5,445,611; this application also claims the benefit of U.S. Provisional Application No. 60/008,043, filed October 30, 1995.

BACKGROUND OF THE INVENTION

15

This invention relates generally to the field of monitoring of analytes in the body and the transdermal delivery of drugs to the body. More particularly, this invention relates to a minimally invasive to non-invasive method of increasing the permeability of the skin through microporation of the stratum corneum, which can be combined with sonic energy, chemical permeation enhancers, pressure, and the like for selectively enhancing outward flux of analytes from the body for monitoring thereof or the delivery of drugs into the body.

20

25

The stratum corneum is chiefly responsible for the well known barrier properties of skin. Thus, it is this layer that presents the greatest barrier to transdermal flux of drugs or other molecules into the body and of analytes out of the body. The stratum corneum, the outer horny layer of the skin, is a complex structure of compact keratinized cell remnants separated by lipid domains. Compared to the oral or gastric mucosa, the stratum corneum is much less permeable to molecules either external or internal to the body. The stratum corneum is formed from keratinocytes, which comprise the majority of epidermal cells, that lose their nuclei and become corneocytes. These dead cells comprise the stratum corneum, which has a thickness of only about 10-30 μm and, as noted above, is a very resistant waterproof membrane that protects the body from invasion by exterior substances and the outward migration of fluids and dissolved molecules. The stratum corneum is continuously renewed by shedding of corneum cells during desquamation and the formation of new corneum cells by the keratinization process.

30

5 The flux of a drug or analyte across the skin can be increased by changing either the resistance (the diffusion coefficient) or the driving force (the gradient for diffusion). Flux may be enhanced by the use of so-called penetration or chemical enhancers. Chemical enhancers are well known in the art and a more detailed description will follow.

 Another method of increasing the permeability of skin to drugs is iontophoresis.

10 Iontophoresis involves the application of an external electric field and topical delivery of an ionized form of drug or an un-ionized drug carried with the water flux associated with ion transport (electro-osmosis). While permeation enhancement with iontophoresis has been effective, control of drug delivery and irreversible skin damage are problems associated with the technique.

15 Sonic energy has also been used to enhance permeability of the skin and synthetic membranes to drugs and other molecules. Ultrasound has been defined as mechanical pressure waves with frequencies above 20 kHz, H. Lutz et al., Manual of Ultrasound 3-12 (1984). Sonic energy is generated by vibrating a piezoelectric crystal or other electromechanical element by passing an alternating current through the material, R. Brucks et al., 6 Pharm. Res. 697 (1989). The use of sonic energy to increase the permeability of the skin to drug molecules has been termed sonophoresis or phonophoresis.

 Although it has been acknowledged that enhancing permeability of the skin should theoretically make it possible to transport molecules from inside the body through the skin to outside the body for collection or monitoring, practicable methods have not been disclosed.

25 U.S. Patent No. 5,139,023 to Stanley et al. discloses an apparatus and method for noninvasive blood glucose monitoring. In this invention, chemical permeation enhancers are used to increase the permeability of mucosal tissue or skin to glucose. Glucose then passively diffuses through the mucosal tissue or skin and is captured in a receiving medium. The amount of glucose in the receiving medium is measured and correlated to determine the blood glucose level. However, as taught in Stanley et al., this method is much more efficient when used on

30 mucosal tissue, such as buccal tissue, which results in detectable amounts of glucose being collected in the receiving medium after a lag time of about 10-20 minutes. However, the

5 method taught by Stanley et al. results in an extremely long lag time, ranging from 2 to 24
hours depending on the chemical enhancer composition used, before detectable amounts of
glucose can be detected diffusing through human skin (heat-separated epidermis) in vitro.
These long lag times may be attributed to the length of time required for the chemical
permeation enhancers to passively diffuse through the skin and to enhance the permeability of
10 the barrier stratum corneum, as well as the length of time required for the glucose to passively
diffuse out through the skin. Thus, Stanley et al. clearly does not teach a method for
transporting blood glucose or other analytes non-invasively through the skin in a manner that
allows for rapid monitoring, as is required for blood glucose monitoring of diabetic patients
and for many other body analytes such blood electrolytes.

15 While the use of sonic energy for drug delivery is known, results have been largely
disappointing in that enhancement of permeability has been relatively low. There is no
consensus on the efficacy of sonic energy for increasing drug flux across the skin. While some
studies report the success of sonophoresis, J. Davick et al., 68 Phys. Ther. 1672 (1988); J.
Griffin et al., 47 Phys. Ther. 594 (1967); J. Griffin & J. Touchstone, 42 Am. J. Phys. Med. 77
20 (1963); J. Griffin et al., 44 Am. J. Phys. Med. 20 (1965); D. Levy et al., 83 J. Clin. Invest.
2074; D. Bommannan et al., 9 Pharm. Res. 559 (1992), others have obtained negative results,
H. Benson et al., 69 Phys. Ther. 113 (1988); J. McElnay et al., 20 Br. J. Clin. Pharmacol.
4221 (1985); H. Pratzel et al., 13 J. Rheumatol. 1122 (1986). Systems in which rodent skin
were employed showed the most promising results, whereas systems in which human skin was
25 employed have generally shown disappointing results. It is well known to those skilled in the
art that rodent skin is much more permeable than human skin, and consequently the above
results do not teach one skilled in the art how to effectively utilize sonophoresis as applied to
transdermal delivery and/or monitoring through human skin.

A significant improvement in the use of ultrasonic energy in the monitoring of
30 analytes and also in the delivery of drugs to the body is disclosed and claimed in copending
applications Serial No. 08/152,442 filed November 15, 1993, now U.S. Patent No. 5,458,140,
and Serial No. 08/152,174 filed December 8, 1993, now U.S. Patent No. 5,445,611, both of

5 which are incorporated herein by reference. In these inventions, the transdermal sampling of
an analyte or the transdermal delivery of drugs, is accomplished through the use of sonic
energy that is modulated in intensity, phase, or frequency or a combination of these parameters
coupled with the use of chemical permeation enhancers. Also disclosed is the use of sonic
energy, optionally with modulations of frequency, intensity, and/or phase, to controllably push
10 and/or pump molecules through the stratum corneum via perforations introduced by needle
puncture, hydraulic jet, laser, electroporation, or other methods.

The formation of micropores (i.e. microporation) in the stratum corneum to enhance
the delivery of drugs has been the subject of various studies and has resulted in the issuance of
patents for such techniques.

15 Jacques et al., 88 J. Invest. Dermatol. 88-93 (1987), teaches a method of administering
a drug by ablating the stratum corneum of a region of the skin using pulsed laser light of
wavelength, pulse length, pulse energy, pulse number, and pulse repetition rate sufficient to
ablate the stratum corneum without significantly damaging the underlying epidermis and then
applying the drug to the region of ablation. This work resulted in the issuance of U.S. Patent
20 4,775,361 to Jacques et al. The ablation of skin through the use of ultraviolet-laser irradiation
was earlier reported by Lane et al., 121 Arch. Dermatol. 609-617 (1985). Jacques et al. is
restricted to use of few wavelengths of light and expensive lasers.

Tankovich, U.S. Patent No. 5,165,418 (hereinafter, "Tankovich '418"), discloses a
method of obtaining a blood sample by irradiating human or animal skin with one or more
25 laser pulses of sufficient energy to cause the vaporization of skin tissue so as to produce a hole
in the skin extending through the epidermis and to sever at least one blood vessel, causing a
quantity of blood to be expelled through the hole such that it can be collected. Tankovich
'418 thus is inadequate for noninvasive or minimally invasive permeabilization of the stratum
corneum such that a drug can be delivered to the body or an analyte from the body can be
30 analyzed.

Tankovich et al., U.S. Patent No. 5,423,803 (hereinafter, "Tankovich '803") discloses
a method of laser removal of superficial epidermal skin cells in human skin for cosmetic

5 applications. The method comprises applying a light-absorbing "contaminant" to the outer
layers of the epidermis and forcing some of this contaminant into the intercellular spaces in the
stratum corneum, and illuminating the infiltrated skin with pulses of laser light of sufficient
intensity that the amount of energy absorbed by the contaminant will cause the contaminant to
explode with sufficient energy to tear off some of the epidermal skin cells. Tankovich '803
10 further teaches that there should be high absorption of energy by the contaminant at the
wavelength of the laser beam, that the laser beam must be a pulsed beam of less than 1 μ s
duration, that the contaminant must be forced into the upper layers of the epidermis, and that
the contaminant must explode with sufficient energy to tear off epidermal cells upon absorption
of the laser energy. This invention also fails to disclose or suggest a method of drug delivery
15 or analyte collection.

Raven et al., WO 92/00106, describes a method of selectively removing unhealthy
tissue from a body by administering to a selected tissue a compound that is highly absorbent of
infrared radiation of wavelength 750-860 nm and irradiating the region with corresponding
infrared radiation at a power sufficient to cause thermal vaporization of the tissue to which the
20 compound was administered but insufficient to cause vaporization of tissue to which the
compound had not been administered. The absorbent compound should be soluble in water or
serum, such as indocyanine green, chlorophyll, porphyrins, heme-containing compounds, or
compounds containing a polyene structure, and power levels are in the range of 50-1000
W/cm² or even higher.

25 Konig et al., DD 259351, teaches a process for thermal treatment of tumor tissue that
comprises depositing a medium in the tumor tissue that absorbs radiation in the red and/or near
red infrared spectral region, and irradiating the infiltrated tissue with an appropriate wavelength
of laser light. Absorbing media can include methylene blue, reduced porphyrin or its
aggregates, and phthalocyanine blue. Methylene blue, which strongly absorbs at 600-700 nm,
30 and a krypton laser emitting at 647 and 676 nm are exemplified. The power level should be at
least 200 mW/cm².

5 It has been shown that by stripping the stratum corneum from a small area of the skin
with repeated application and removal of cellophane tape to the same location one can easily
collect arbitrary quantities of interstitial fluid, which can then be assayed for a number of
analytes of interest. Similarly, the 'tape-stripped' skin has also been shown to be permeable to
the transdermal delivery of compounds into the body. Unfortunately, 'tape-stripping' leaves a
10 open sore which takes weeks to heal, and for this, as well as other reasons, is not considered as
an acceptable practice for enhancing transcutaneous transport in wide applications.

 As discussed above, it has been shown that pulsed lasers, such as the excimer laser
operating at 193 nm, the erbium laser operating near 2.9 μm or the CO₂ laser operating at 10.2
 μm , can be used to effectively ablate small holes in the human stratum corneum. These laser
15 ablation techniques offer the potential for a selective and potentially non-traumatic method for
opening a delivery and/or sampling hole through the stratum corneum. However, due to the
prohibitively high costs associated with these light sources, there have been no commercial
products developed based on this concept. The presently disclosed invention, by defining a
method for directly conducting thermal energy into the stratum corneum with very tightly
20 defined spatial and temporal resolution, makes it possible to produce the desired micro-ablation
of the stratum corneum using very low cost energy sources.

 In view of the foregoing problems and/or deficiencies, the development of a method
for safely enhancing the permeability of the skin for minimally invasive or noninvasive
monitoring of body analytes in a more rapid time frame would be a significant advancement in
25 the art. It would be another significant advancement in the art to provide a method of
minimally invasively or non-invasively enhancing the transdermal flux rate of a drug into a
selected area of an individual's body.

BRIEF SUMMARY OF THE INVENTION

30 An object of the invention is to minimize the barrier properties of the stratum
corneum using poration to controllably collect analytes from within the body through
perforations in the stratum corneum to enable the monitoring of these analytes.

5 It is also an object of the invention to provide a method of monitoring selected analytes in the body through micropores in the stratum corneum in combination with sonic energy, permeation enhancers, pressure gradients, and the like.

 Another object of the invention is to provide a method for controlling transdermal flux rates of drugs or other molecules into the body and, if desired, into the bloodstream through
10 minute perforations in the stratum corneum.

 It is still another object of the invention to provide a method of delivering drugs into the body through micropores in the stratum corneum in combination with sonic energy, permeation enhancers, pressure gradients, and the like.

 These and other objects may be accomplished by providing a method for monitoring
15 the concentration of an analyte in an individual's body comprising the steps of enhancing the permeability of the stratum corneum of a selected area of the individual's body surface to the analyte by means of

- (a) porating the stratum corneum of the selected area by means that form a micro-pore in the stratum corneum without causing serious damage to the underlying tissues, thereby
20 reducing the barrier properties of the stratum corneum to the withdrawal of the analyte;
- (b) collecting a selected amount of the analyte; and
- (c) quantitating the analyte collected.

 In one preferred embodiment, the method further comprises applying sonic energy to the porated selected area at a frequency in the range of about 5 kHz to 100 MHz, wherein the
25 sonic energy is modulated by means of a member selected from the group consisting of frequency modulation, amplitude modulation, phase modulation, and combinations thereof. In another preferred embodiment, the method further comprises contacting the selected area of the individual's body with a chemical enhancer with the application of the sonic energy to further enhance analyte withdrawal.

 Porating of the stratum corneum is accomplished by means selected from the group
30 consisting of (a) ablating the stratum corneum by contacting a selected area, up to about 1000 μm across, of the stratum corneum with a heat source such that the temperature of

5 tissue-bound water and other vaporizable substances in the selected area is elevated above the vaporization point of the water and other vaporizable substances thereby removing the stratum corneum in the selected area; (b) puncturing the stratum corneum with a micro-lancet calibrated to form a micropore of up to about 1000 μm in diameter; (d) ablating the stratum corneum by focusing a tightly focused beam of sonic energy onto the stratum corneum; (d) 10 hydraulically puncturing the stratum corneum with a high pressure jet of fluid to form a micropore of up to about 1000 μm in diameter and (e) puncturing the stratum corneum with short pulses of electricity to form a micropore of up to about 1000 μm in diameter.

One preferred embodiment of thermally ablating the stratum corneum comprises treating at least the selected area with an effective amount of a dye that exhibits strong 15 absorption over the emission range of a pulsed light source and focusing the output of a series of pulses from the pulsed light source onto the dye such that the dye is heated sufficiently to conductively transfer heat to the stratum corneum to elevate the temperature of tissue-bound water and other vaporizable substances in the selected area above the vaporization point of the water and other vaporizable substances. Preferably, the pulsed light source emits at a 20 wavelength that is not significantly absorbed by skin. For example, the pulsed light source can be a laser diode emitting in the range of about 630 to 1550 nm, a laser diode pumped optical parametric oscillator emitting in the range of about 700 and 3000 nm, or a member selected from the group consisting of arc lamps, incandescent lamps, and light emitting diodes. A sensing system for determining when the barrier properties of the stratum corneum have been 25 surmounted can also be provided. One preferred sensing system comprises light collection means for receiving light reflected from the selected area and focusing the reflected light on a photodiode, a photodiode for receiving the focused light and sending a signal to a controller wherein the signal indicates a quality of the reflected light, and a controller coupled to the photodiode and to the pulsed light source for receiving the signal and for shutting off the 30 pulsed light source when a preselected signal is received.

In another preferred embodiment, the method further comprises cooling the selected area of stratum corneum and adjacent skin tissues with cooling means such that said selected

5 area and adjacent skin tissues are in a selected precooled, steady state, condition prior to poration.

In still another preferred embodiment, the method comprises ablating the stratum corneum such that interstitial fluid exudes from the micropores, collecting the interstitial fluid, and analyzing the analyte in the collected interstitial fluid. After the interstitial fluid is
10 collected, the micropore can be sealed by applying an effective amount of energy from the laser diode or other light source such that interstitial fluid remaining in the micropore is caused to coagulate. Preferably, vacuum is applied to the porated selected area to enhance collection of interstitial fluid.

In yet another preferred embodiment, the method comprises, prior to porating the
15 stratum corneum, illuminating at least the selected area with unfocused light from the pulsed light source such that the selected area illuminated with the light is sterilized.

Another preferred method of porating the stratum corneum comprises contacting the selected area with a metallic wire such that the temperature of the selected area is raised from ambient skin temperature to greater than 100°C within about 10 to 50 ms and then returning
20 the temperature of the selected area to approximately ambient skin temperature within about 30 to 50 ms, wherein this cycle of raising the temperature and returning to approximately ambient skin temperature is repeated a number of time effective for reducing the barrier properties of the stratum corneum. Preferably, the step of returning to approximately ambient skin temperature is carried out by withdrawing the wire from contact with the stratum corneum. It
25 is also preferred to provide means for monitoring electrical impedance between the wire and the individual's body through the selected area of stratum corneum and adjacent skin tissues and means for advancing the position of the wire such that as the ablation occurs with a concomitant reduction in resistance, the advancing means advances the wire such that the wire is in contact with the stratum corneum during heating of the wire. Further, it is also preferred
30 to provide means for withdrawing the wire from contact with the stratum corneum, wherein the monitoring means is capable of detecting a change in impedance associated with contacting an epidermal layer underlying the stratum corneum and sending a signal to the withdrawing means

5 to withdrawn the wire from contact with the stratum corneum. The wire can be heated by an
ohmic heating element, can have a current loop having a high resistance point wherein the
temperature of the high resistance point is modulated by passing a modulated electrical current
through said current loop to effect the heating, or can be positioned in a modulatable
alternating magnetic field of an excitation coil such that energizing the excitation coil with
10 alternating current produces eddy currents sufficient to heat the wire by internal ohmic losses.

A method for enhancing the transdermal flux rate of an active permeant into a
selected area of an individual's body comprising the steps of enhancing the permeability of the
stratum corneum layer of the selected area of the individual's body surface to the active
permeant by means of

- 15 (a) porating the stratum corneum of the selected area by means that form a micro-pore
in the stratum corneum without causing serious damage to the underlying tissues and thereby
reduce the barrier properties of the stratum corneum to the flux of the active permeant; and
(b) contacting the porated selected area with a composition comprising an effective
amount of the permeant such that the flux of the permeant into the body is enhanced.

20 In a preferred embodiment, the method further comprises applying sonic energy to the
porated selected area for a time and at an intensity and a frequency effective to create a fluid
streaming effect and thereby enhance the transdermal flux rate of the permeant into the body.

A method is also provided for applying a tattoo to a selected area of skin on an
individual's body surface comprising the steps of:

- 25 (a) porating the stratum corneum of the selected area by means that form a
micro-pore in the stratum corneum without causing serious damage to the underlying tissues
and thereby reduce the barrier properties of the stratum corneum to the flux of a permeant; and
(b) contacting the porated selected area with a composition comprising an effective
amount of a tattooing ink as a permeant such that the flux of said ink into the body is enhanced.

30 A method is still further provided for reducing a temporal delay in diffusion of an
analyte from blood of an individual to said individual's interstitial fluid in a selected area of
skin comprising applying means for cooling to said selected area of skin.

5 A method is yet further provided for reducing evaporation of interstitial fluid and the vapor pressure thereof, wherein said interstitial fluid is being collected from a micropore in a selected area of stratum corneum of an individual's skin, comprising applying means for cooling to said selected area of skin.

10 BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

FIG. 1 shows a schematic representation of a system for delivering laser diode light and monitoring the progress of poration.

FIG. 2 shows a schematic representation of a closed-loop feedback system for monitoring poration.

15 FIG. 3A shows a schematic representation of an optical poration system comprising a cooling device.

FIG. 3B shows a top view of a schematic representation of an illustrative cooling device according to FIG. 3A.

20 FIG. 4 shows a schematic representation of an ohmic heating device with a mechanical actuator.

FIG. 5 shows a schematic representation of a high resistance current loop heating device.

FIG. 6 shows a schematic representation of a device for modulating heating using inductive heating.

25 FIG. 7 shows a schematic representation of a closed loop impedance monitor using changes in impedance to determine the extent of poration.

FIGS. 8A-D show cross sections of human skin treated with copper phthalocyanine and then subjected, respectively, to 0, 1, 5, and 50 pulses of 810 nm light with an energy density of 4000 J/cm² for a pulse period of 20 ms.

30 FIGS. 9-11 show graphic representations of temperature distribution during simulated thermal poration events using optical poration.

5 FIGS. 12 and 13 show graphic representations of temperature as a function of time in the stratum corneum and viable epidermis, respectively, during simulated thermal poration events using optical poration.

 FIGS. 14-16 show graphic representations of temperature distribution, temperature as a function of time in the stratum corneum, and temperature as a function of time in the viable
10 epidermis, respectively, during simulated thermal poration events using optical poration wherein the tissue was cooled prior to poration.

 FIGS. 17-19 show graphic representations of temperature distribution, temperature as a function of time in the stratum corneum, and temperature as a function of time in the viable
15 epidermis, respectively, during simulated thermal poration events wherein the tissue was heated with a hot wire.

 FIGS. 20-22 show graphic representations of temperature distribution, temperature as a function of time in the stratum corneum, and temperature as a function of time in the viable
20 epidermis, respectively, during simulated thermal poration events wherein the tissue was heated with a hot wire and the tissue was cooled prior to poration.

 FIGS. 23 and 24 show graphic representations of temperature distribution and temperature as a function of time in the stratum corneum, respectively, during simulated thermal poration events wherein the tissue is heated optically according to the operating parameters of Tankovich '803.

 FIG. 25 shows a graphic representation of interstitial fluid (ISF; ○) and blood (*)
25 glucose levels as a function of time.

 FIG. 26 shows a scatter plot representation of the difference term between the ISF glucose and the blood glucose data of FIG. 25.

 FIG. 27 shows a histogram of the relative deviation of the ISF to the blood glucose levels from FIG. 25.

30 FIG. 28 shows a cross section of an illustrative delivery apparatus for delivering a drug to a selected area on an individual's skin.

5 FIGS. 29A-C show graphic representations of areas of skin affected by delivery of lidocaine to selected areas where the stratum corneum is porated (FIGS. 29A-B) or not porated (FIG. 29C).

 FIG. 30 shows a plot comparing the amount of interstitial fluid harvested from micropores with suction alone (o) and with a combination of suction and ultrasound (*).

10 FIGS. 31, 32, and 33 show a perspective view of an ultrasonic transducer/vacuum apparatus for harvesting interstitial fluid, a cross section view of the same apparatus, and cross sectional schematic view of the same apparatus, respectively.

 FIGS. 34A-B show a top view of a handheld ultrasonic transducer and a side view of the spatulate end thereof, respectively.

15

DETAILED DESCRIPTION

 Before the present methods for permeabilizing the stratum corneum for facilitating transdermal drug delivery and analyte sampling are disclosed and described, it is to be understood that this invention is not limited to the particular configurations, process steps, and materials disclosed herein as such configurations, process steps, and materials may vary somewhat. It is also to be understood that the terminology employed herein is used for the purpose of describing particular embodiments only and is not intended to be limiting since the scope of the present invention will be limited only by the appended claims and equivalents thereof.

25 It must be noted that, as used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to a method for delivery of "a drug" includes reference to delivery of a mixture of two or more drugs, reference to "an analyte" includes reference to one or more of such analytes, and reference to "a permeation enhancer" includes reference to a mixture of two or more permeation enhancers.

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 In describing and claiming the present invention, the following terminology will be used in accordance with the definitions set out below.

5 As used herein, "poration," "microporation," or any such similar term means the formation of a small hole or pore in the stratum corneum in a selected area of the skin of an individual to lessen the barrier properties of this layer of the skin to the passage of analytes from below the skin surface for analysis or the passage of active permeants or drugs into the body for therapeutic purposes. Preferably the hole or pore will be no larger than about 1 mm
10 in diameter, and more preferably no larger than about 100 μm in diameter, and will extend into the stratum corneum sufficiently to break the barrier properties of this layer without adversely affecting the underlying tissues.

As used herein "ablation" means the controlled removal of cells caused by kinetic energy released when the vaporizable components of the cells have been heated to the point
15 that vaporization occurs and the resulting rapid expansion of volume due to this phase change causes cells and possibly some adjacent cells to be "blown away" from the ablation site.

As used herein "puncture" or "micro-puncture" means the use of mechanical, hydraulic, or electrical means to perforate the stratum corneum.

20 To the extent that "ablation" and "puncture" accomplish the same purpose of poration, i.e. the creating a hole or pore in the stratum corneum without significant damage to the underlying tissues, these terms may be used interchangeably.

As used herein, "penetration enhancement" or "permeation enhancement" means an increase in the permeability of skin to a drug, analyte, dye, stain, or other chemical molecule
25 (also called "permeant"), i.e., so as to increase the rate at which a drug, analyte, or chemical molecule permeates the stratum corneum and facilitates the poration of the stratum corneum, the withdrawal of analytes out through the stratum corneum or the delivery of drugs through the stratum corneum and into the underlying tissues. The enhanced permeation effected through the use of such enhancers can be observed, for example, by observing diffusion of a
30 dye, as a permeant, through animal or human skin using a diffusion apparatus.

As used herein, "chemical enhancer," "penetration enhancer," "permeation enhancer," and the like includes all enhancers that increase the flux of a permeant, analyte, or other

5 molecule across the skin, and is limited only by functionality. In other words, all cell envelope disordering compounds and solvents and any other chemical enhancement agents are intended to be included.

As used herein, "dye," "stain," and the like shall be used interchangeably and refer to a biologically suitable chromophore that exhibits strong absorption at the emission range of a pulsed light source used to ablate tissues of the stratum corneum to form micropores therein.

10 As used herein, "transdermal" or "percutaneous" means passage of a permeant into and through the skin to achieve effective therapeutic blood levels or deep tissue levels of a drug, or the passage of a molecule present in the body ("analyte") out through the skin so that the analyte molecule may be collected on the outside of the body.

15 As used herein, the term "permeant," "drug," or "pharmacologically active agent" or any other similar term means any chemical or biological material or compound suitable for transdermal administration by the methods previously known in the art and/or by the methods taught in the present invention, that induces a desired biological or pharmacological effect, which may include but is not limited to (1) having a prophylactic effect on the organism and preventing an undesired biological effect such as preventing an infection, (2) alleviating a condition caused by a disease, for example, alleviating pain or inflammation caused as a result of disease, and/or (3) either alleviating, reducing, or completely eliminating the disease from the organism. The effect may be local, such as providing for a local anaesthetic effect, or it may be systemic. This invention is not drawn to novel permeants or to new classes of active agents. Rather it is limited to the mode of delivery of agents or permeants that exist in the state of the art or that may later be established as active agents and that are suitable for delivery by the present invention. Such substances include broad classes of compounds normally delivered into the body, including through body surfaces and membranes, including skin. In general, this includes but is not limited to: antiinfectives such as antibiotics and antiviral agents; analgesics and analgesic combinations; anorexics; antihelminthics; antiarthritics; antiasthmatic agents; anticonvulsants; antidepressants; antidiabetic agents; antidiarrheals; antihistamines; antiinflammatory agents; antimigraine preparations;

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5 antinauseants; antineoplastics; antiparkinsonism drugs; antipruritics; antipsychotics; antipyretics; antispasmodics; anticholinergics; sympathomimetics; xanthine derivatives; cardiovascular preparations including potassium and calcium channel blockers, beta-blockers, alpha-blockers, and antiarrhythmics; antihypertensives; diuretics and antidiuretics; vasodilators including
10 general coronary, peripheral and cerebral; central nervous system stimulants; vasoconstrictors; cough and cold preparations, including decongestants; hormones such as estradiol and other steroids, including corticosteroids; hypnotics; immunosuppressives; muscle relaxants; parasympatholytics; psychostimulants; sedatives; and tranquilizers. By the method of the present invention, both ionized and nonionized drugs may be delivered, as can drugs of either high or low molecular weight.

15 As used herein, an "effective" amount of a pharmacologically active agent means a sufficient amount of a compound to provide the desired local or systemic effect and performance at a reasonable benefit/risk ratio attending any medical treatment. An "effective" amount of a permeation or chemical enhancer as used herein means an amount selected so as to provide the desired increase in skin permeability and the desired depth of penetration, rate
20 of administration, and amount of drug delivered.

As used herein, "carriers" or "vehicles" refer to carrier materials without significant pharmacological activity at the quantities used that are suitable for administration with other pharmaceutically active materials, and include any such materials known in the art, e.g., any liquid, gel, solvent, liquid diluent, solubilizer, or the like, that is nontoxic at the quantities
25 employed and does not interact with the drug to be administered in a deleterious manner. Examples of suitable carriers for use herein include water, mineral oil, silicone, inorganic gels, aqueous emulsions, liquid sugars, waxes, petroleum jelly, and a variety of other oils and polymeric materials.

As used herein, a "biological membrane" is intended to mean a membrane material
30 present within a living organism that separates one area of the organism from another and, in many instances, that separates the organism from its outer environment. Skin and mucous membranes are thus included.

5 As used herein, "individual" refers to both a human and an animal, to which the present invention may be applied.

As used herein, "analyte" means any chemical or biological material or compound suitable for passage through a biological membrane by the technology taught in this present invention, or by technology previously known in the art, of which an individual might want to know the concentration or activity inside the body. Glucose is a specific example of an
10 analyte because it is a sugar suitable for passage through the skin, and individuals, for example those having diabetes, might want to know their blood glucose levels. Other examples of analytes include, but are not limited to, such compounds as sodium, potassium, bilirubin, urea, ammonia, calcium, lead, iron, lithium, salicylates, and the like.

15 As used herein, "transdermal flux rate" is the rate of passage of any analyte out through the skin of an individual, human or animal, or the rate of passage of any drug, pharmacologically active agent, dye, or pigment in and through the skin of an individual, human or animal.

As used herein, the terms "intensity amplitude," "intensity," and "amplitude" are used
20 synonymously and refer to the amount of energy being produced by the sonic energy system.

As used herein, "frequency modulation" or "sweep" means a continuous, graded or stepped variation in the amplitude or frequency of ultrasound in a given time period. A frequency modulation is a graded or stepped variation in frequency in a given time period, for example 5.4-5.76 MHz in 1 sec., or 5-10 MHz in 0.1 sec., or 10-5 MHz in 0.1 sec., or any
25 other frequency range or time period that is appropriate to a specific application. A complex modulation can include varying both the frequency and intensity simultaneously. For example, FIGS. 4A and 4B of U.S. Patent No. 5,458,140 could, respectively, represent amplitude and frequency modulations being applied simultaneously to a single sonic energy transducer.

As used herein "phase modulation" means the timing of the signal has been changed
30 relative to its initial state shown in Fig. 4C of U.S. Patent No. 5,458,140. The frequency and amplitude of the signal can remain the same. A phase modulation can be implemented with a

5 variable delay such as to selectively retard or advance the signal temporarily in reference to its previous state, or to another signal.

The sonic energy, in its various applications such as with frequency, intensity or phase modulation, or combinations thereof and the use of chemical enhancers combined with modulated sonic energy, as described herein, can vary over a frequency range of between about
10 5 kHz to 100 MHz, with a range of between about 20 kHz and 30 MHz being preferred.

As used herein, "non-invasive" means not requiring the entry of a needle, catheter, or other invasive medical instrument into a part of the body.

As user herein, "minimally invasive" refers to the use of mechanical, hydraulic, or electrical means that invade the stratum corneum to create a small hole or micropore without
15 causing substantial damage to the underlying tissues.

Means for Poration of the Stratum Corneum

The formation of a micropore in the stratum corneum can be accomplished by various state of the art means as well as certain means disclosed herein that are improvements thereof.

20 The use of laser ablation as described by Jacques et al. in U.S. Patent 4,775,361 and by Lane et al., supra, certainly provide one means for ablating the stratum corneum using an excimer laser. At 193 nm wavelength, and 14 ns pulsewidth, it was found that about 0.24 to 2.8 μm of stratum corneum could be removed by each laser pulse at radiant exposure of between about 70 and 480 mJ/cm^2 . As the pulse energy increases, more tissue is removed
25 from the stratum corneum and fewer pulses are required for complete poration of this layer. The lower threshold of radiant exposure that must be absorbed by the stratum corneum within the limit of the thermal relaxation time to cause suitable micro-explosions that result in tissue ablation is about 70 mJ/cm^2 within a 50 millisecond (ms) time. In other words, a total of 70 mJ/cm^2 must be delivered within a 50 ms window. This can be done in a single pulse of 70
30 mJ/cm^2 or in 10 pulses of 7 mJ/cm^2 , or with a continuous illumination of 1.4 watts/ cm^2 during the 50 ms time. The upper limit of radiant exposure is that which will ablate the stratum corneum without damage to underlying tissue and can be empirically determined from the light

5 source, wavelength of light, and other variables that are within the experience and knowledge of one skilled in this art.

By "deliver" is meant that the stated amount of energy is absorbed by the tissue to be ablated. At the excimer laser wavelength of 193 nm, essentially 100% absorption occurs within the first 1 or 2 μm of stratum corneum tissue. Assuming the stratum corneum is about 10 20 μm thick, at longer wavelengths, such as 670 nm, only about 5% of incident light is absorbed within the 20 μm layer. This means that about 95% of the high power beam passes into the tissues underlying the stratum corneum where it will likely cause significant damage.

The ideal is to use only as much power as is necessary to perforate the stratum corneum without causing bleeding, thermal, or other damage to underlying tissues from which 15 analytes are to be extracted or drugs or other permeants delivered.

It would be beneficial to use sources of energy more economical than energy from excimer lasers. Excimer lasers, which emit light at wavelengths in the far UV region, are much more expensive to operate and maintain than, for example, diode lasers that emit light at wavelengths in visible and IR regions (600 to 1800 nm). However, at the longer wavelengths, 20 the stratum corneum becomes increasingly more transparent and absorption occurs primarily in the underlying tissues.

The present invention facilitates a rapid and painless method of eliminating the barrier function of the stratum corneum to facilitate the transcutaneous transport of therapeutic 25 substances into the body when applied topically or to access the analytes within the body for analysis. The method utilizes a procedure which begins with the contact application of a small area heat source to the targeted area of the stratum corneum.

The heat source must have several important properties, as will now be described. First, the heat source must be sized such that contact with the skin is confined to a small area, 30 typically about 1 to 1000 μm in diameter. Second, it must have the capability to modulate the temperature of the stratum corneum at the contact point from ambient skin surface temperature levels (33°C) to greater than 123°C and then return to approximately ambient skin temperature

5 with cycle times to minimize collateral damage to viable tissues and sensation to the subject individual. This modulation can be created electronically, mechanically, or chemically.

Additionally, an inherent depth limiting feature of the microporation process can be facilitated if the heat source has both a small enough thermal mass and limited energy source to elevate its temperature such that when it is placed in contact with tissues with more than
10 30% water content, the thermal dispersion in these tissues is sufficient to limit the maximum temperature of the heat source to less than 100°C. This feature effectively stops the thermal vaporization process once the heat probe had penetrated through the stratum corneum into the lower layers of the epidermis.

With the heat source placed in contact with the skin, it is cycled through a series of
15 one or more modulations of temperature from an initial point of ambient skin temperature to a peak temperature in excess of 123°C to approximately ambient skin temperature. To minimize or eliminate the subject's sensory perception of the microporation process, these pulses are limited in duration, and the interpulse spacing is long enough to allow cooling of the viable tissue layers in the skin, and most particularly the enervated dermal tissues, to achieve a mean
20 temperature of less than about 45°C. These parameters are based on the thermal time constants of the viable epidermal tissues (roughly 30-80 ms) located between the heat probe and the enervated tissue in the underlying dermis. The result of this application of pulsed thermal energy is that enough energy is conducted into the stratum corneum within the tiny target spot that the local temperature of this volume of tissue is elevated sufficiently higher than the
25 vaporization point of the tissue-bound water content in the stratum corneum. As the temperature increases above 100°C, the water content of the stratum corneum (typically 5% to 15%) within this localized spot, is induced to vaporize and expand very rapidly, causing a vapor-driven removal of those corneocytes in the stratum corneum located in proximity to this vaporization event. U.S. Patent No. 4,775,361 teaches that a stratum corneum temperature of
30 123°C represents a threshold at which this type of flash vaporization occurs. As subsequent pulses of thermal energy are applied, additional layers of the stratum corneum are removed until a micropore is formed through the stratum corneum down to the next layer of the

5 epidermis, the stratum lucidum. By limiting the duration of the heat pulse to less than one thermal time constant of the epidermis and allowing any heat energy conducted into the epidermis to dissipate for a sufficiently long
10 enough time, the elevation in temperature of the viable layers of the epidermis is minimal. This allows the entire microporation process to take place without any sensation to the subject and no damage to the underlying and surrounding tissues.

The present invention comprises a method for painlessly creating microscopic holes, i.e. micropores, from about 1 to 1000 μm across, in the stratum corneum of human skin. The key to successfully implementing this method is the creation of an appropriate thermal energy source, or heat probe, which is held in contact with the stratum corneum. The principle
15 technical challenge in fabricating an appropriate heat probe is designing a device that has the desired contact with the skin and that can be thermally modulated at a sufficiently high frequency.

It is possible to fabricate an appropriate heat probe by topically applying to the stratum corneum a suitable light-
20 absorbing compound, such as a dye or stain, selected because of its ability to absorb light at the wavelength emitted by a selected light source. In this instance, the selected light source may be a laser diode emitting at a wavelength which would not normally be absorbed by the skin tissues. By focusing the light source to a small spot on the surface of the topical layer of the dye, the targeted area can be temperature modulated by varying the intensity of the light
25 flux focused on it. It is possible to utilize the energy from laser sources emitting at a longer wavelength than an excimer laser by first topically applying to the stratum corneum a suitable light-absorbing compound, such as a dye or stain, selected because of its ability to absorb light at the wavelength emitted by the laser source. The same concept can be applied at any wavelength and one must only choose an appropriate dye or stain and optical wavelength. One
30 need only look to any reference manual to find which suitable dyes and wavelength of the maximum absorbance of that dye. One such reference is Green, The Sigma-Aldrich Handbook of Stains, Dyes and Indicators, Aldrich Chemical Company, Inc. Milwaukee, Wisconsin (1991).

5 For example, copper phthalocyanine (Pigment Blue 15; CPC) absorbs at about 800 nm; copper
phthalocyanine tetrasulfonic acid (Acid Blue 249) absorbs at about 610 nm; and Indocyanine
Green absorbs at about 775 nm; and Cryptocyanine absorbs at about 703 nm. CPC is
particularly well suited for this embodiment for the following reasons: it is a very stable and
inert compound, already approved by the FDA for use as a dye in implantable sutures; it
10 absorbs very strongly at wavelengths from 750 nm to 950 nm, which coincide well with
numerous low cost, solid state emitters such as laser diodes and LEDs, and in addition, this
area of optical bandwidth is similarly not absorbed directly by the skin tissues in any
significant amount; CPC has a very high vaporization point (>550C in a vacuum) and goes
directly from a solid phase to a vapor phase with no liquid phase; CPC has a relatively low
15 thermal diffusivity constant, allowing the light energy focused on it to selectively heat only
that area directly in the focal point with very little lateral spreading of the 'hot-spot' into the
surrounding CPC thereby assisting in the spatial definition of the contact heat-probe.

The purpose of this disclosure is not to make an exhaustive listing of suitable dyes or
stains because such may be easily ascertained by one skilled in the art from data readily
20 available.

The same is true for any desired particular pulsed light source. For example, this
method may be implemented with a mechanically shuttered, focused incandescent lamp as the
pulse light source. Various catalogs and sales literature show numerous lasers operating in the
near UV, visible and near IR range. Representative lasers are Hamamatsu Photonic Systems
25 Model PLP-02 which operates at a power output of 2×10^{-4} J, at a wavelength of 415 nm;
Hamamatsu Photonic Systems Model PLP-05 which operates at a power output of 15 J, at a
wavelength of 685 nm; SDL, Inc., SDL-3250 Series pulsed laser which operates at a power
output of 2×10^6 J at a wavelength of about 800-810 nm; SDL, Inc., Model SDL-8630 which
operates at a power output of 500 mW at a wavelength of about 670 nm; Uniphase Laser
30 Model AR-081-15000 which operates at a power output of 15,000 mW at a wavelength of
790-830 nm; Toshiba America Electronic Model TOLD9150 which operates at a power output

5 of 30 mW at a wavelength of 690 nm; and LiCONIX, Model Diolite 800-50 which operates at a power 50 mW at a wavelength of 780 nm.

For purposes of the present invention a pulsed laser light source can emit radiation over a wide range of wavelengths ranging from between about 100 nm to 12,000 nm. Excimer lasers typically will emit over a range of between about 100 to 400 nm. Commercial excimer
10 lasers are currently available with wavelengths in the range of about 193 nm to 350 nm. Preferably a laser diode will have an emission range of between about 380 to 1550 nm. A frequency doubled laser diode will have an emission range of between about 190 and 775 nm. Longer wavelengths ranging from between about 1300 and 3000 nm may be utilized using a laser diode pumped optical parametric oscillator. It is expected, given the amount of research
15 taking place on laser technology, that these ranges will expand with time.

Delivered or absorbed energy need not be obtained from a laser as any source of light, whether it is from a laser, a short arc lamp such as a xenon flashlamp, an incandescent lamp, a light-emitting diode (LED), the sun, or any other source may be used. Thus, the particular instrument used for delivering electromagnetic radiation is less important than the wavelength
20 and energy associated therewith. Any suitable instrument capable of delivering the necessary energy at suitable wavelengths, *i.e.* in the range of about 100 nm to about 12,000 nm, can be considered within the scope of the invention. The essential feature is that the energy must be absorbed by the light-absorbing compound to cause localized heating thereof, followed by conduction of sufficient heat to the tissue to be ablated within the timeframe allowed.

25 In one illustrative embodiment, the heat probe itself is formed from a thin layer, preferably about 5 to 1000 μm thick, of a solid, non-biologically active compound, applied topically to a selected area of an individual's skin that is large enough to cover the site where a micropore is to be created. The specific formulation of the chemical compound is chosen such that it exhibits high absorption over the spectral range of a light source selected for providing
30 energy to the light-absorbing compound. The probe can be, for example, a sheet of a solid compound, a film treated with a high melting point absorbing compound, or a direct application of the light-absorbing compound to the skin as a precipitate or as a suspension in a

5 carrier. Regardless of the configuration of the light-absorbing heat probe, it must exhibit a low enough lateral thermal diffusion coefficient such that any local elevations of temperature will remain spatially defined and the dominant mode of heat loss will be via direct conduction into the stratum corneum through the point of contact between the skin and the probe.

10 The required temperature modulation of the probe can be achieved by focusing a light source onto the light-absorbing compound and modulating the intensity of this light source. If the energy absorbed within the illuminated area is sufficiently high, it will cause the light absorbing compound to rapidly heat up. The amount of energy delivered, and subsequently both the rate of heating and peak temperature of the light-absorbing compound at the focal point, can be easily modulated by varying the pulse width and peak power of the light source.

15 In this embodiment, it is only the small volume of light-absorbing compound heated up by the focused, incident optical energy that forms the heat probe, additional light absorbing compound which may have been applied over a larger area than the actual poration site is incidental. By using a solid phase light-absorbing compound with a relatively high melting point, such as copper phthalocyanine (CPC), which remains in its solid phase up to a temperature of greater

20 than 550°C, the heat probe can be quickly brought up to a temperature of several hundred degrees C, and still remain in contact with the skin, allowing this thermal energy to be conducted into the stratum corneum. In addition, this embodiment comprises choosing a light source with an emission spectrum where very little energy would normally be absorbed in the skin tissues.

25 Once the targeted area has the light-absorbing compound topically positioned on it, the heat probe is formed when the light source is activated with the focal waist of the beam positioned to be coincident with the surface of the treated area. The energy density of light at the focal waist and the amount of absorption taking place within the light-absorbing compound are set to be sufficient to bring the temperature of the light-absorbing compound, within the

30 area of the small spot defined by the focus of the light source, to greater than 123°C within a few milliseconds. As the temperature of the heat probe rises, conduction into the stratum corneum delivers energy into these tissues, elevating the local temperature of the stratum

5 corneum. When enough energy has been delivered into this small area of stratum corneum to cause the local temperature to be elevated above the boiling point of the water contained in these tissues, a flash vaporization of this water takes place, ablating the stratum corneum at this point.

10 By turning the light source on and off, the temperature of the heat probe can be rapidly modulated and the selective ablation of these tissues can be achieved, allowing a very precisely dimensioned hole to be created, which selectively penetrates only through the first 10 to 30 μm of skin.

15 An additional feature of this embodiment is that by choosing a light source that would normally have very little energy absorbed by the skin or underlying tissues, and by designing the focusing and delivery optics to have a sufficiently high numerical aperture, the small amount of delivered light that does not happen to get absorbed in the heat probe itself, quickly diverges as it penetrates deep into the body. Since there is very little absorption at the delivered wavelengths, essentially no energy is delivered to the skin directly from the light source. This three dimensional dilution of coupled energy in the tissues due to beam
20 divergence and the low level of absorption in the untreated tissue results in a completely benign interaction between the light beam and the tissues, with no damage being done thereby.

25 In one preferred embodiment of the invention, a laser diode is used as the light source with an emission wavelength of $800 \pm 30 \text{ nm}$. A heat-probe can be formed by topical application of a transparent adhesive tape that has been treated on the adhesive side with a 0.5 cm spot formed from a deposit of finely ground copper phthalocyanine (CPC). The CPC exhibits extremely high absorption coefficients in the 800 nm spectral range, typically absorbing more than 95% of the radiant energy from a laser diode.

30 FIG. 1 shows a system 10 for delivering light from such a laser diode to a selected area of an individual's skin and for monitoring the progress of the poration process. The system comprises a laser diode 14 coupled to a controller 18, which controls the intensity, duration, and spacing of the light pulses. The laser diode emits a beam 22 that is directed to a collection lens or lenses 26, which focuses the beam onto a mirror 30. The beam is then

5 reflected by the mirror to an objective lens or lenses 34, which focuses the beam at a
preselected point 38. This preselected point corresponds with the plane of an xyz stage 42 and
the objective hole 46 thereof, such that a selected area of an individual's skin can be irradiated.
The xyz stage is connected to the controller such that the position of the xyz stage can be
controlled. The system also comprises a monitoring system comprising a CCD camera 50
10 coupled to a monitor 54. The CCD camera is confocally aligned with the objective lens such
that the progress of the poration process can be monitored visually on the monitor.

In another illustrative embodiment of the invention, a system of sensing photodiodes
and collection optics that have been confocally aligned with the ablation light source is
provided. FIG. 2 shows a sensor system 60 for use in this embodiment. The system
15 comprises a light source 64 for emitting a beam of light 68, which is directed through a
delivery optics system 72 that focuses the beam at a preselected point 76, such as the surface
of an individual's skin 80. A portion of the light contacting the skin is reflected, and other
light is emitted from the irradiated area. A portion of this reflected and emitted light passes
through a filter 84 and then through a collection optics system 88, which focuses the light on a
20 photodiode 92. A controller 96 is coupled to both the laser diode and the photodiode for,
respectively, controlling the output of the laser diode and detecting the light that reaches the
photodiode. Only selected portions of the spectrum emitted from the skin pass through the
filter. By analyzing the shifts in the reflected and emitted light from the targeted area, the
system has the ability to detect when the stratum corneum has been breached, and this
25 feedback is then used to control the light source, deactivating the pulses of light when the
microporation of the stratum corneum is achieved. By employing this type of active closed
loop feedback system, a self regulating, universally applicable device is obtained that produces
uniformly dimensioned micropores in the stratum corneum, with minimal power requirements,
regardless of variations from one individual to the next.

30 In another illustrative embodiment, a cooling device is incorporated into the system
interface to the skin. FIG. 3A shows an illustrative schematic representation thereof. In this
system 100, a light source 104 (coupled to a controller 106) emits a beam of light 108, which

5 passes through and is focused by a delivery optics system 112. The beam is focused by the delivery optics system to a preselected point 116, such as a selected area of an individual's skin 120. A cooling device 124, such as a Peltier device or other means of chilling, contacts the skin to cool the surface thereof. In a preferred embodiment of the cooling device 124 (FIG. 3B), there is a central hole 128 through which the beam of focused light passes to
10 contact the skin. Referring again to FIG. 3A, a heat sink 132 is also preferably placed in contact with the cooling device. By providing a cooling device with a small hole in its center coincident with the focus of the light, the skin tissues in the general area where the poration is to be created may be pre-cooled to 5°C to 10°C. This pre-cooling allows a greater safety margin for the system to operate in that the potential sensations to the user and the possibility
15 of any collateral damage to the epidermis directly below the poration site are reduced significantly from non-cooled embodiment. Moreover, for monitoring applications, pre-cooling minimizes evaporation of interstitial fluid and can also provide advantageous physical properties, such as decreased surface tension of such interstitial fluid. Still further, cooling the tissue is known to cause a localized increase in blood flow in such cooled tissue, thus
20 promoting diffusion of analytes from the blood into the interstitial fluid.

The method can also be applied for other micro-surgery techniques wherein the light-absorbing compound/heat-probe is applied to the area to be ablated and then the light source is used to selectively modulate the temperature of the probe at the selected target site, affecting the tissues via the vaporization-ablation process produced.

25 A further feature of the invention is to use the light source to help seal the micropore after its usefulness has passed. Specifically, in the case of monitoring for an internal analyte, a micropore is created and some amount of interstitial fluid is extracted through this opening. After a sufficient amount of interstitial fluid had been collected, the light source is reactivated at a reduced power level to facilitate rapid clotting or coagulation of the interstitial fluid within
30 the micropore. By forcing the coagulation or clotting of the fluid in the pore, this opening in the body is effectively sealed, thus reducing the risk of infection. Also, the use of the light source itself for both the formation of the micropore and the sealing thereof is an inherently

5 sterile procedure, with no physical penetration into the body by any device or apparatus. Further, the thermal shock induced by the light energy kills any microbes that may happen to be present at the ablation site.

10 This concept of optical sterilization can be extended to include an additional step in the process wherein the light source is first applied in an unfocused manner, covering the target area with an illuminated area that extends 100 μm or more beyond the actual size of the micropore to be produced. By selecting the area over which the unfocused beam is to be applied, the flux density can be correspondingly reduced to a level well below the ablation threshold but high enough to effectively sterilize the surface of the skin. After a sufficiently long exposure of the larger area, either in one continuous step or in a series of pulses, to the sterilizing beam, the system is then configured into the sharply focused ablation mode and the optical microporation process begins.

15 Another illustrative embodiment of the invention is to create the required heat probe from a metallic solid, such as a small diameter wire. As in the previously described embodiment, the contacting surface of the heat probe must be able to have its temperature modulated from ambient skin temperatures (33°C) to temperatures greater than 123°C, within 20 the required time allowed of, preferably, between about 1 to 50 ms at the high temperature (on-time) and at least about 10 to 50 ms at the low temperature (off-time). In particular, being able to modulate the temperature up to greater than 150°C for an "on" time of around 5 ms and an off time of 50 ms produces very effective thermal ablation with little or no sensation to 25 the individual.

Several methods for modulating the temperatures of the wire heat probe contact area may be successfully implemented. For example, a short length of wire may be brought up to the desired high temperature by an external heating element such as an ohmic heating element used in the tip of a soldering iron. FIG. 4 shows an ohmic heating device 140 with a 30 mechanical actuator. The ohmic heating device comprises an ohmic heat source 144 coupled to a wire heat probe 148. The ohmic heat source is also coupled through an insulating mount 152 to a mechanical modulation device 156, such as a solenoid. In this configuration, a steady

5 state condition can be reached wherein the tip of the wire probe will stabilize at some
equilibrium temperature defined by the physical parameters of the structure, i.e., the
temperature of the ohmic heat source, the length and diameter of the wire, the temperature of
the air surrounding the wire, and the material of which the wire is comprised. Once the
desired temperature is achieved, the modulation of the temperature of the selected area of an
10 individual's skin 160 is effected directly via the mechanical modulation device to alternatively
place the hot tip of the wire in contact with the skin for, preferably, a 5 ms on-time and then
withdraw it into the air for, preferably, a 50 ms off-time.

Another illustrative example (FIG. 5), shows a device 170 comprising a current source
174 coupled to a controller 178. The current source is coupled to a current loop 182
15 comprising a wire 186 formed into a structure such that it presents a high resistance point.
Preferably, the wire is held on a mount 190, and an insulator 194 separates different parts of
the current loop. The desired modulation of temperature is then achieved by merely
modulating the current through the wire. If the thermal mass of the wire element is
appropriately sized and the heat sinking provided by the electrodes connecting it to the current
20 source is sufficient, the warm-up and cool-down times of the wire element can be achieved in
a few milliseconds. Contacting the wire with a selected area of skin 198 heats the stratum
corneum to achieve the selected ablation.

In FIG. 6 there is shown still another illustrative example of porating the stratum
corneum with a hot wire. In this system 200, the wire 204 can be positioned within a
25 modulatable alternating magnetic field formed by a coil of wire 208, the excitation coil. By
energizing the alternating current in the excitation coil by means of a controller 212 coupled
thereto, eddy currents can be induced in the wire heat probe of sufficient intensity that it will
be heated up directly via the internal ohmic losses. This is essentially a miniature version of
an inductive heating system commonly used for heat treating the tips of tools or inducing out-
30 gassing from the electrodes in vacuum or flash tubes. The advantage of the inductive heating
method is that the energy delivered into the wire heat probe can be closely controlled and
modulated easily via the electronic control of the excitation coil. If the thermal mass of the

5 wire probe itself, and the thermal mass of the stratum corneum in contact with the tip of the probe are known, controlling the inductive energy delivered can produce very precise control of the temperature at the contact point 216 with the skin 220. Because the skin tissue is essentially non-magnetic at the lower frequencies at which inductive heating can be achieved, if appropriately selected frequencies are used in the excitation coil, then this alternating
10 electromagnetic field will have no effect on the skin tissues.

If a mechanically controlled contact modulation is employed, an additional feature may be realized by incorporating a simple closed loop control system wherein the electrical impedance between the probe tip and the subject's skin is monitored. In this manner, the position of the probe can be brought into contact with the subject's skin, indicated by the step-
15 wise reduction in resistance once contact is made, and then held there for the desired "on-time," after which it can be withdrawn. Several types of linear actuators are suitable for this form of closed loop control, such as a voice-coil mechanism, a simple solenoid, a rotary system with a cam or bell-crank, and the like. The advantage is that as the thermal ablation progresses, the position of the thermal probe tip can be similarly advanced into the skin,
20 always ensuring good a contact to facilitate the efficient transfer of the required thermal energy. Also, the change in the conductivity properties of the stratum corneum and the epidermis can be used to provide an elegant closed loop verification that the poration process is complete, i.e., when the resistance indicates that the epidermis has been reached, it is time to stop the poration process.

25 FIG. 7 shows an illustrative example of such a closed loop impedance monitor. In this system 230, there is an ohmic heat source 234 coupled to a wire heat probe 238. The heat source is mounted through an insulating mount 242 on a mechanical modulator 246. A controller 250 is coupled to the wire and to the skin 254, wherein the controller detects changes in impedance in the selected area 258 of skin, and when a predetermined level is
30 obtained the controller stops the poration process.

Along the same line as hydraulic poration means are microlancets adapted to just penetrate the stratum corneum for purposes of administering a permeant, such as a drug.

5 through the pore formed or to withdraw an analyte through the pore for analysis. Such a device is considered to be "minimally invasive" as compared to devices and/or techniques which are non-invasive. The use of micro-lancets that penetrate below the stratum corneum for withdrawing blood are well known. Such devices are commercially available from manufacturers such as Becton-Dickinson and Lifescan and can be utilized in the present
10 invention by controlling the depth of penetration. As an example of a micro-lancet device for collecting body fluids, reference is made to Erickson et al., International Published PCT [REDACTED] This application shows a device for penetration into the dermal layer of the skin, without penetration into subcutaneous tissues, to collect body fluids for monitoring, such as for blood glucose levels.

15 Poration of stratum corneum can also be accomplished using sonic means. Sonic-poration is a variation of the optical means described above except that, instead of using a light source, a very tightly focused beam of sonic energy is delivered to the area of the stratum corneum to be ablated. The same levels of energy are required, i.e. a threshold of 70 mJ/cm²/50 ms still must be absorbed. The same pulsed focused ultrasonic transducers as
20 described in parent applications Serial Nos. 08/152,442 and 08/152,174 can be utilized to deliver the required energy densities for ablation as are used in the delivery of sonic energy which is modulated in intensity, phase, or frequency or a combination of these parameters for the transdermal sampling of an analyte or the transdermal delivery of drugs. This has the advantage of allowing use of the same transducer to push a drug through the stratum corneum
25 or pull a body fluid to the surface for analysis to be used to first create a micro-pore.

Additionally, electroporation or short bursts or pulses of electrical current can be delivered to the stratum corneum with sufficient energy to form micropores [REDACTED]

[REDACTED]
[REDACTED] Thus, a person of skill in this art can select an instrument and
30 conditions for use thereof without undue experimentation according to the guidelines provided herein.

5 The micropores produced in the stratum corneum by the methods of the present invention allow high flux rates of large molecular weight therapeutic compounds to be delivered transdermally. In addition, these non-traumatic microscopic openings into the body allow access to various analytes within the body, which can be assayed to determine their internal concentrations.

10 Example 1

In this example, skin samples were prepared as follows. Epidermal membrane was separated from human cadaver whole skin by the heat-separation method of Klingman and Christopher, 88 Arch. Dermatol. 702 (1963), involving the exposure of the full thickness skin to a temperature of 60°C for 60 seconds, after which time the stratum corneum and part of the epidermis (epidermal membrane) were gently peeled from the dermis.

Example 2

Heat separated stratum corneum samples prepared according to the procedure of Example 1 were cut into 1 cm² sections. These small samples were then attached to a glass cover slide by placing them on the slide and applying a pressure sensitive adhesive backed disk with a 6 mm hole in the center over the skin sample. The samples were then ready for experimental testing. In some instances the skin samples were hydrated by allowing them to soak for several hours in a neutral buffered phosphate solution or pure water.

As a test of these untreated skin samples, the outputs of several different infrared laser diodes, emitting at roughly 810, 905, 1480 and 1550 nanometers were applied to the sample. The delivery optics were designed to produce a focal waist 25 μm across with a final objective have a numerical aperture of 0.4. The total power delivered to the focal point was measured to be between 50 and 200 milliwatts for the 810 and 1480 nm laser diodes, which were capable of operating in a continuous wave (CW) fashion. The 905 and 1550 nm laser diodes were designed to produce high peak power pulses roughly 10 to 200 nanoseconds long at repetition rates up to 5000 Hz. For the pulsed lasers the peak power levels were measured to be 45 watts at 905 nm and 3.5 watts at 1550 nm.

5 Under these operating conditions, there was no apparent effect on the skin samples from any of the lasers. The targeted area was illuminated continuously for 60 seconds and then examined microscopically, revealing no visible effects. In addition, the sample was placed in a modified Franz cell, typically used to test transdermal delivery systems based on chemical permeation enhancers, and the conductivity from one side of the membrane to the other was measured both before and after the irradiation by the laser and showed no change. Based on these tests which were run on skin samples from four different donors, it was concluded that at these wavelengths the coupling of the optical energy into the skin tissue was so small that no effects are detectable.

15

Example 3

To evaluate the potential sensation to a living subject when illuminated with optical energy under the conditions of Example 2, six volunteers were used and the output of each laser source was applied to their fingertips, forearms, and the backs of their hands. In the cases of the 810, 905 and 1550 nm lasers, the subject was unable to sense when the laser was turned on or off. In the case of the 1480 nm laser, there was a some sensation during the illumination by the 1480 nm laser operating at 70 mW CW, and a short while later a tiny blister was formed under the skin due to the absorption of the 1480 nm radiation by one of the water absorption bands. Apparently the amount of energy absorbed was sufficient to induce the formation of the blister, but was not enough to cause the ablative removal of the stratum corneum. Also, the absorption of the 1480 nm light occurred predominantly in the deeper, fully hydrated (85% to 90% water content) tissues of the epidermis and dermis, not the relatively dry (10% to 15% water content) tissue of the stratum corneum.

25

Example 4

30 Having demonstrated the lack of effect on the skin in its natural state (Example 3), a series of chemical compounds was evaluated for effectiveness in absorbing the light energy and then transferring this absorbed energy, via conduction, into the targeted tissue of the stratum

5 corneum. Compounds tested included India ink; "SHARPIE" brand indelible black, blue, and red marking pens; methylene blue; fuschian red; epolite #67, an absorbing compound developed for molding into polycarbonate lenses for protected laser goggles; tincture of iodine; iodine-polyvinylpyrrolidone complex ("BETADINE"); copper phthalocyanine; and printers ink.

Using both of the CW laser diodes described in Example 2, positive ablation results
10 were observed on the in vitro samples of heat-separated stratum corneum prepared according to Example 1 when using all of these products, however some performed better than others. In particular the copper phthalocyanine (CPC) and the epolite #67 were some of the most effective. One probable reason for the superior performance of the CPC is its high boiling point of greater the 500°C and the fact that it maintains its solid phase up to this temperature.

15

Example 5

As copper phthalocyanine has already been approved by the FDA for use in implantable sutures, and is listed in the Merck index as a rather benign and stabile molecule in regard to human biocompatibility, the next step taken was to combine the topical application
20 of the CPC and the focused light source to the skin of healthy human volunteers. A suspension of finely ground CPC in isopropyl alcohol was prepared. The method of application used was to shake the solution and then apply a small drop at the target site. As the alcohol evaporated, a fine and uniform coating of the solid phase CPC was then left on the surface of the skin.

25 The apparatus show in FIG. 1 was then applied to the site, wherein the CPC had been topically coated onto the skin, by placing the selected area of the individual's skin against a reference plate. The reference plate consists of a thin glass window roughly 3 cm X 3 cm, with a 4 mm hole in the center. The CPC covered area was then positioned such that it was within the central hole. A confocal video microscope (FIG. 1) was then used to bring the
30 surface of the skin into sharp focus. Positioning the skin to achieve the sharpest focus on the video system also positioned it such that the focal point of the laser system was coincident with the surface of the skin. The operator then activated the pulses of laser light while

5 watching the effects at the target site on the video monitor. The amount of penetration was
stimulated visually by the operator by gauging the amount of defocusing of the laser spot in the
micropore as the depth of the micropore increased, and this can be dynamically corrected by
the operator, essentially following the ablated surface down into the tissues by moving the
position of the camera/laser source along the "z" axis, into the skin. At the point when the
10 stratum corneum had been removed down to the epidermis, the appearance of the base of the
hole changed noticeably, becoming much wetter and shinier. Upon seeing this change, the
operator deactivated the laser. In many instances, depending on the state of hydration of the
subject as well as other physiological conditions, a dramatic outflow of interstitial fluid
occurred in response to the barrier function of the stratum corneum being removed over this
15 small area. The video system was used to record this visual record of the accessibility of
interstitial fluid at the poration site.

Example 6

The procedure of Example 5 was followed except that the CPC was applied to a
20 transparent adhesive tape, which was then caused to adhere to a selected site on the skin of an
individual. The results were substantially similar to those of Example 5.

Example 7

Histology experiments were performed on cadaver skin according to methods well
25 known in the art to determine ablation threshold parameters for given dye mixtures and
collateral damage information. The top surface of the skin sample was treated with a solution
of copper phthalocyanine (CPC) in alcohol. After the alcohol evaporated, a topical layer of
solid phase CPC was distributed over the skin surface with a mean thickness of 10 to 20 μm .
FIG. 8A shows a cross-section of full thickness skin prior to the laser application, wherein the
30 CPC layer 270, stratum corneum 274, and underlying epidermal layers 278 are shown. FIG.
8B shows the sample after a single pulse of 810 nm light was applied to an 80 μm diameter
circle with an energy density of 4000 J/cm², for a pulse period of 20 ms. It is noteworthy that

5 there was still a significant amount of CPC present on the surface of the stratum corneum even in the middle of the ablated crater 282. It should also be noted that laboratory measurements indicate that only about 10% of the light energy incident on the CPC is actually absorbed, with the other 90% being reflected or backscattered. Thus the effective energy flux being delivered to the dye layer which could cause the

10 desired heating is only about 400 J/cm². 8C shows the sample after 5 pulses of 810 nm light were applied, wherein the stratum corneum barrier was removed with no damage to the underlying tissue. These results are a good representation of the "ideal" optically modulated thermal ablation performance. FIG. 8D shows the sample after 50 pulses were applied. Damaged tissue 286 was present in the epidermal layers due to carbonization of non ablated

15 tissue and thermal denaturing of the underlying tissue. FIGS. 8A-8C show separations between the stratum corneum and the underlying epidermal layers due to an artifact of dehydration, freezing, and preparations for imaging.

Example 8

20 To examine the details of the thermal ablation mechanism, a mathematical model of the skin tissues was constructed upon which various different embodiments of the thermal ablation method could be tried. This model computes the temperature distribution in a layered semi-infinite medium with a specified heat flux input locally on the surface and heat removal from the surface some distance away, i.e. convection is applied between the two. The

25 axisymmetric, time-dependent diffusion equation is solved in cylindrical coordinates using the alternating-direction-implicit (ADI) method. (Note: Constant Temp. B.C. is applied on lower boundary to serve as $z \rightarrow \infty$; and zero radial heat flux is applied on max radial boundary to serve as $r \rightarrow \infty$). The layers are parallel to the surface and are defined as: (1) dye; (2) stratum corneum; (3) underlying epidermis; and (4) dermis. The depth into the semi-infinite medium and thermal properties, density (ρ), specific heat (c), and conductivity (k) must be specified

30 for each layer.

5 First, a heat-transfer coefficient, h , on the skin is computed based on the "steady," "1-D," temperature distribution determined by the ambient air temperature, skin surface temperature, and dermis temperature. It is assumed that there is no dye present and provides "h" on the skin surface. The program then allows one to use this "h" on the dye layer surface or input another desired "h" for the dye surface. Next, the "steady" temperature distribution is
10 computed throughout all layers (including the dye layer) using the specified "h" at the dye surface. This temperature distribution is the initial condition for the time-dependent heating problem. This constitutes the "m-file" initial.m. The program then solves for the time-dependent temperature distribution by marching in time, computing and displaying the temperature field at each step.

15 Each embodiment of the method described herein, for which empirical data have been collected, has been modeled for at least one set of operational parameters, showing how stratum corneum ablation can be achieved in a precise and controllable fashion. The output of the simulations is presented graphically in two different formats: (1) a cross-sectional view of the skin showing the different tissue layers with three isotherms plotted on top of this view
20 which define three critical temperature thresholds, and (2) two different temperature -vs- time plots, one for the point in the middle of the stratum corneum directly beneath the target site, and the second for the point at the boundary of the viable cell layers of the epidermis and the underside of the stratum corneum. These plots show how the temperature at each point varies with time as the heat pulses are applied as if one could implant a microscopic thermocouple
25 into the tissues. In addition, the application of this model allows investigation of the parametric limits within which the method can be employed to set the outer limits for two important aspects of the methods performance. First, general cases are presented cases that define the envelope within which the method can be employed without causing pain or undesired tissue damage.

30 For any given heat source, as described in the several different embodiments of the invention, there is a point at which the effect on the subject's skin tissues becomes non-optimal in that the subject perceives a pain sensation, or that the viable cells in the underlying

5 epidermis and/or dermis sustain temperatures, which if maintained for a long enough duration, will render damage to these tissues. Accordingly, a test simulation was run using the optically heated topical copper phthalocyanine (CPC) dye embodiment as a baseline method to establish how the thermal time constants of the different skin tissue layers essentially define a window within which the method can be employed without pain or damage to adjacent tissue layers.

10 FIGS. 9 and 10 show schematic cross-sectional views of the skin and the topical dye layer. In each figure, three distinct isotherms are displayed: (1) 123°C, the point at which vaporization of the water in the tissue produces an ablation of the tissue; (2) 70°C, the point at which viable cells will be damaged if this temperature is maintained for several seconds; and (3) 45°C, the average point at which a sensation of pain will be perceived by the subject. This pain threshold is described in several basic physiology texts, but experience shows this threshold to be somewhat subjective. In fact, in repeated tests on the same individual, different poration sites within a few millimeters of each other can show significantly different amounts of sensation, possibly due to the proximity to a nerve ending in relationship to the poration site.

20 The dimensions on the graphs show the different layers of the dye and skin, as measured in μm , with flat boundaries defining them. Whereas the actual skin tissues have much more convoluted boundaries, in a mean sense for the dimensions involved, the model provides a good approximation of the thermal gradients present in the actual tissues. The dimensions used in this, and all subsequent simulations, for the thicknesses of the CPC dye layer and the various skin layers are as follows: dye, 10 μm ; stratum corneum, 30 μm ; underlying epidermis, 70 μm ; and dermis, 100 μm .

25 Additional conditions imposed on the model for this particular simulation are shown in the following tables:

5

10

15

| Table 1 | |
|--|-----------------------------------|
| Initial Conditions for Finite Difference Thermal Model | |
| Ambient Air Temperature | Ta = 20°C |
| Skin Surface Temperature | Ts = 30°C |
| Dermis Temperature | Td = 37°C |
| Dye Vaporization Temperature | Tvap = 550°C |
| S.C. Vaporization Temperature | Tc1 = 123°C |
| Tissue Damage Temperature | Tc2 = 70°C |
| "Pain" Temperature | Tc3 = 45°C |
| Radius of Irradiated Area | R _{bot} = 30 µm |
| Energy Density Applied | FLUX = 400 Joules/cm ² |

Table 2

20

| Parameter | Dye | S.C. | Epidermis | Dermis |
|----------------------|---------|--------|-----------|---------|
| Thermal Conductivity | 0.00046 | .00123 | 0.00421 | 0.00421 |
| Density | 0.67 | 1.28 | 1.09 | 1.09 |
| Specific Heat | 0.8 | 1.88 | 3.35 | 3.35 |

When these simulations are run, the following conservative assumptions are imposed:

25

1. While some portion of the stratum corneum may be shown as having a temperature already exceeded the ablation threshold for thermal vaporization of the water content, this event is not modeled, and the subsequent loss of heat energy in the tissues due to this vaporization is not factored into the simulation. This will cause a slight elevation in the temperatures shown in the underlying tissues from that point on in the simulation run.

30

2. Similarly, when some portion of the copper phthalocyanine (CPC) dye layer is shown to have reached its vaporization point of 550°C, this event is not modeled, but the temperature is merely hard-limited to this level. This will also cause a slight elevation of the subsequent temperatures in the underlying layers as the simulation progresses.

35

Even with these simplifications used in the model, the correlation between the predicted performance and the empirically observed performance based on both clinical studies and histological studies on donor tissue samples is remarkable. The key data to note in FIGS. 9 and 10 are the length of time which the heat pulse is applied, and the location of the three different threshold temperatures displayed by the isotherms.

In FIG. 9, with a pulse length of 21 milliseconds, the 70°C isotherm just crosses the boundary separating the stratum corneum and the viable cell layers in the epidermis. In in

5 vitro studies on donor skin samples under these conditions, fifty pulses of thermal energy delivered 50 milliseconds apart cause detectable damage to this top layer of living cells (see FIG. 8D). However, it was also shown in the in vitro studies that five pulses of heat energy at these same operating parameters, did not produce any significant damage to these tissues. It seems reasonable that even though the nominal damage threshold may have been exceeded, at least in a transient sense, this temperature must be maintained for some cumulative period of time to actually cause any damage to the cells. Nevertheless, the basic information presented by the simulation is that if one keeps the "on-time" of the heat pulse to less than 10 milliseconds with the flux density of 400 Joules/cm², then no damage to the living cells in the underlying epidermis will be sustained, even though the ablation threshold isotherm has been moved well into the stratum corneum. 15 ~~by the simulation is that if one keeps the "on-time" of the heat pulse to less than 10 milliseconds with the flux density of 400 Joules/cm², then no damage to the living cells in the underlying epidermis will be sustained, even though the ablation threshold isotherm has been moved well into the stratum corneum.~~ ~~corneum can be achieved without any damage to the adjacent cells in the underlying epidermis~~ (see FIG. 8C). This is possible in large part due to the significantly different thermal diffusivities of these two tissues layers. That is, the stratum corneum, containing only about 20 10% to 20% water content, has a much lower thermal conductivity constant, 0.00123 J/(S*cm*K), than the .00421 J/(S*cm*K) of the epidermis. This allows the temperature to build up in the stratum corneum, while maintaining a tight spatial definition, to the point at which ablation will occur.

In FIG. 10, the same simulation scenario started in the damage threshold critical point run illustrated in FIG. 9 is carried out farther in time. By leaving the heat pulse on for 58 25 milliseconds at the same flux density of 400 Joules/cm² within the 60 μ m diameter circle of dye being heated, the pain sensory isotherm at 45°C just enters the enervated layer of skin comprised by the dermis. In addition, the damage threshold isotherm moves significantly farther into the epidermal layer than where it was shown to be in FIG. 9. Relating this 30 simulation to the numerous clinical studies conducted with this method, an excellent verification of the model's accuracy is obtained in that the model shows almost exactly the duration of 'on-time' that the heat probe can be applied to the skin before the individual feels it. In clinical tests, a controllable pulse generator was used to set the "on-time" and "off-time" of a series of light pulses applied to the topical layer of copper phthalocyanine (CPC) dye on 35 the skin. While maintaining a constant "off-time" of 80 milliseconds, the "on-time" was gradually increased until the subject reported a mild "pain" sensation. Without exception, all of the subjects involved in these studies, reported the first "pain" at an "on-time" of between 45 and 60 milliseconds, very close to that predicted by the model. In addition, the site-to-site variability mentioned previously as regards the sensation of "pain" was noted in these clinical 40 studies. Accordingly, what is reported as "pain" is the point at which the first unambiguous

5 sensation is noticeable. At one site this may be reported as pain, whereas at an adjacent site the same subject may report this as merely "noticeable."

One element of this clinical research is the realization that even at the same site, a non-uniform pulse-train of heat pulses may work with the subject's psycho-physiological neuro-perception to cause a genuine reduction in perceived sensation. For example, a series of shorter length heat pulses can be used to saturate the neurons in the area, momentarily depleting the neuro-transmitters available at this synaptic junction and therefore limiting the ability to send a "pain" message. This then allows a longer pulse following these short pulses to be less noticeable than if it were applied at the beginning of the sequence. Accordingly, a series of experiments was conducted with some arbitrarily created pulse trains, and the results were consistent with this hypothesis. An analogy for this situation might be found in the perception when one first steps into a very hot bath that is painful at first, but quickly becomes tolerable as one acclimates to the heat sensation.

Example 9

20 An object of this invention is to achieve a painless, micro-poration of the stratum comeum without causing any significant damage to the adjacent viable tissues. As described in the simulation illustrated in Example 8 and FIGS. 9-10, a boundary appears to exist for any given flux density of thermal energy within the ablation target spot within which the micro-poration can be achieved in just such a painless and non-traumatic manner. Both the in vivo and in vitro studies have shown that this is the case, and this has permitted development through empirical methods of some operational parameters that appear to work very well. The following set of simulations shows how the method works when these specific parameters are used.

30 In the first case, a pulse train of ten pulses, 10 milliseconds "on-time" separated by 10 milliseconds "off-time" is applied to the CPC-covered skin. FIG. 11 shows the final temperature distribution in the skin tissues immediately after this pulse train has ended. As can be seen, the isotherms representing the three critical temperature thresholds show that stratum comeum ablation has been achieved, with no sensation present in the dermal layer nerves and very little cross-over of the damage threshold into the viable cells of the underlying epidermis. As mentioned previously, it appears that to actually do permanent cell damage, the epidermal cells must not only be heated up to a certain point, but they also must be held at this temperature for some period of time, generally thought to be about five seconds. FIGS. 12 and 13 show the temperature of the stratum comeum and the viable epidermis, respectively, as a function of time, showing heating during the "on-time" and cooling during the "off-time" for the entire ten cycles. Relating this simulation to the in vivo studies conducted, note that in better than 90% of the poration attempts with the system parameters set to match the

5 simulation, effective poration of the stratum corneum was achieved without pain to the subject, and in subsequent microscopic examination of the poration site several days later, no noticeable damage to the tissues was apparent. The in vitro studies conducted on whole thickness donor skin samples were also consistent with the model's prediction of behavior.

10

Example 10

In conducting both the empirical in vivo studies, and these simulations, it appears that prechilling ~~of the skin prior to the micro-poration process for reducing the~~
~~probability of pain or damage to adjacent tissue.~~ In practice, this can easily be achieved using a simple cold-plate placed against the skin prior to the poration process. For example,
15 applying a Peltier cooled plate to the 1 cm diameter circle surrounding the poration target site, with the plate held at roughly 5°C for a few seconds, significantly reduces the temperature of the tissues. A schematic illustration of an experimental device used for this purpose in the laboratory is shown in FIGS. 3A-B. By applying exactly the same ten-cycle pulse train as used in the run illustrated in Example 9, one can see, by comparing FIG. 11 to FIG. 14, FIG.
20 12 to FIG. 15, and FIG. 13 to FIG. 16, how much improvement can be made in the control of the temperature penetration into the skin tissues. Once again, the relatively low thermal diffusivity and specific heat of the stratum corneum as compared to the epidermis and dermis is advantageous. Once cooled, the highly hydrated tissues of the epidermis and dermis require a much larger thermal energy input to elevate their temperatures, whereas the stratum corneum,
25 with its relatively dry makeup, can quickly be heated up to the ablation threshold.

Example 11

Once the basic thermal conduction mechanism of delivering the energy into the skin tissues underlying the effective painless ablation and micro-poration of the stratum corneum is
30 understood, several different specific methods to achieve the required rapid temperature modulations of the contact point can be conceived, such as the hot wire embodiments illustrated in FIGS. 4-7.

A basic embodiment, as described herein, uses an Ohmic heating element (FIG. 4), such as the tip of a small cordless soldering iron, with a suitably sized, relatively non-reactive,
35 wire wrapped around it with a short amount of the wire left to protrude away from the body of the heater. When electricity is applied with a constant current source, the heater will come up to some temperature and within a few seconds, achieve a steady state with the convection losses to the surrounding air. Similarly, the wire, which is a part of this thermal system, will reach a steady state such that the very tip of the wire can be raised to almost any arbitrary
40 temperature, up to roughly 1000°C with these types of components. The tip can be sized to give exactly the dimension micropore desired.

5 In the laboratory, tungsten wires with a diameter of 80 μm attached to the replaceable
tip of a "WAHL" cordless soldering iron with approximately 2 mm of wire protruding from
the tip have been utilized. With a thermocouple, the temperature of the tip has been measured
at its steady state, and it has been noted that by varying the constant current settings, steady
10 state temperatures of greater than 700°C can easily be reached. To achieve the desired
modulation, a low mass, fast response electromechanical actuator was coupled to the tip such
that the position of the wire could be translated linearly more than 2 mm at up to a 200 Hz
rate. Then, by mounting the entire apparatus on a precision stage, this vibrating tip could very
controllably be brought into contact with the skin surface in a manner where it was only in
15 contact for less than 10 milliseconds at a time, the "on-time," while an "off-time" of arbitrarily
long periods could be achieved by setting the pulse generator accordingly. These in vivo
studies showed that the poration could actually be achieved before the subject being porated
even knew that the tip of the wire was being brought into contact with the skin.

To compare the performance of this embodiment to the optically heated topical CPC
dye embodiment, the following simulations were run according to the procedure of Example 8.
20 Essentially, by only varying the initial conditions, the hot wire embodiment can be run with
the identical simulation code. Because the contact with the wire occurs essentially instantly,
there is no time dependent build-up of heat in the CPC dye layer and when the wire is
physically removed from contact with the skin, there is a no residual heat still left on the
surface as there is with the heated CPC dye layer. Also, as the wire itself defines the area
25 targeted for ablation/micro-poration, there should be no lateral diffusion of thermal energy
prior to its application to the stratum corneum. The comparative performances of the "hot-
wire" embodiment are shown in FIGS. 17-19.

Example 12

30 In this example, the procedure of Example 11 was followed except that the skin was
pre-cooled according to the procedure of Example 10. Similarly, pre-cooling the target site
yields similarly positive results with the "hot-wire" embodiment. The results of the pre-cooled
simulation of the "hot-wire" approach are shown in FIGS. 20-22.

Example 13

35 As discussed in the background introduction of this disclosure, the Tankovich '803
patent appears at first glance to be similar to the presently claimed invention. In this example,
the simulation model was set up with the operating parameters specified in Tankovich '803,
i.e. a pulse width of 1 μs and a power level of 40,000,000 W/cm². FIGS. 23 and 24 show that
40 under these conditions no portion of the stratum corneum reaches the threshold for flash
vaporization of water, 123°C, and thus no ablation/microporation of the stratum corneum

5 occurs. In practice, applying this type of high peak power, short duration pulse to the topical dye layer merely vaporizes the dye off of the surface of the skin with no effect on the skin. This example, thus, demonstrates that the conditions specified by Tankovich '803 are inoperative in the presently claimed invention.

10 Example 14

In this example, interstitial fluid obtained after porating the skin according to the procedure of Example 6 was collected and analyzed to determine the glucose concentration thereof. Data were obtained on four non-diabetic subjects and six type I diabetic subjects undergoing a glucose load test. Subject's ages ranged from 27 to 43. The goal of the study was to examine the utility of the method for painlessly harvesting enough interstitial fluid (ISF) from the subjects to allow the ISF samples to be assayed for glucose content, and then compare these concentrations to the glucose level presenting in the subject's whole blood.

15 All subjects had both the blood and ISF glucose assays performed with the "ELITE" system from Miles-Bayer. All ten subjects underwent identical measurement protocols, with adjustments being made regarding the glucose load and insulin shot for those subjects with insulin dependent diabetes.

20 The basic design of the study was to recruit a modest number of volunteers, some with diabetes and some without diabetes, from which a series of sample pairs of ISF and whole blood were drawn every 3 to 5 minutes throughout the 3 to 4 hour duration of the study period. Both the blood and the ISF samples were assayed for glucose and the statistical relationship between the blood glucose levels and the interstitial fluid determined. To examine the hypothesized temporal lag of the ISF glucose levels as compared to the whole blood glucose levels, the study subjects were induced to exhibit a significant and dynamic change in their glucose levels. This was accomplished by having each subject fast for 12 hours prior to beginning the test and then giving the subject a glucose load after his or her baseline glucose levels have been established via a set of three fasting blood and ISF glucose levels. After the baseline levels had been established, the subjects were given a glucose load in the form of sweet juice based on the following guidelines:

35 i. For the control subjects, the glucose load was calculated based on a .75 gram glucose per pound of body weight.

40 ii. For the subjects with insulin dependent diabetes the glucose load was 50 grams of glucose. In addition, immediately after taking the glucose load the diabetic subjects will self inject their normal morning dose of fast acting insulin. In the case where the diabetic subject presents with fasting glucose levels above 300 mg/dL, they were asked to give themselves their insulin injection first, and the

5 glucose load was provided after their blood glucose levels have dropped to below 120 mg/dL.

Each subject recruited was first given a complete description of the study in the "Informed Consent" document which they were required to understand and sign before they were officially enrolled into the program. Upon acceptance, they completed a medical history questionnaire. The detailed clinical procedure implemented was:

- 10 (a) Subject fasted from 9:00 p.m. the night before the study visit, consuming only water. No caffeine, cigarettes, fruit juice were allowed during this period.
- (b) Subject arrived at the testing facility by 9:00 a.m. the next day.
- 15 (c) Subject was seated in a reclining chair provided for the subject to relax in throughout the study procedure.
- (d) Both whole blood and ISF samples were taken at three to five minute intervals beginning upon the subject's arrival and continuing for the next three to four hours. The duration over which the data were collected was based on when the subject's blood glucose levels had returned to the normal range and stabilized after the glucose load. The ISF samples were harvested using the optical poration, ISF pumping method, described in more detail below. Each ISF sample was roughly 5 uL by volume to ensure a good fill of the ELITE test strip. The blood samples were obtained via a conventional finger prick lancet. Both the ISF and the blood samples were immediately assayed for glucose with the ELITE home glucometer system from Miles-Bayer. To improve the estimate of the 'true' blood glucose levels, two separate ELITE assays were be done on each finger stick sample.
- 20 (e) To facilitate the continued collection of the ISF from the same site throughout the entire data collection phase for a given individual, a 5 by 5 matrix of twenty five micropores was created on the subject's upper forearm, each micropore being between 50 and 80 μ m across and spaced 300 μ m apart. A 30 mm diameter teflon disk with a 6 mm hole in the center was attached to the subject's forearm with a pressure sensitive adhesive and positioned such that the 6 mm center hole was located over the 5 by 5 matrix of micro-pores. This attachment allowed a convenient method by which a small suction hose could be connected, applying a mild vacuum (10 to 12 inches of Hg) to the porated area to induce the ISF to flow out of the body through the micropores. The top of the teflon disk was fitted with a clear glass window allowing the operator to directly view the micro-porated skin beneath it. When a 5 uL bead of ISF was formed on the surface of the skin, it could easily be ascertained by visually monitoring the site through this window. This level of vacuum created a nominal pressure gradient of around 5 pounds/square inch (PSI). Without the micropores, no ISF whatsoever could be drawn from the subject's body using only the mild vacuum.
- 25
- 30
- 35
- 40

5 (f) After the first three sample pairs have been drawn, the subject was given a glucose load in the form of highly sweetened orange juice. The amount of glucose given was 0.75 grams per pound of body weight for the nondiabetic subjects and 50 grams for the diabetic subjects. The diabetic subjects also self administered a shot of fast acting insulin, (regular) with the dosage appropriately calculated, based on this 50 gram level of glucose
10 concurrent with the ingestion of the glucose load. With the normal 1.5 to 2.5 hour lag between receiving an insulin shot and the maximum effect of the shot, the diabetic subjects were expected to exhibit an upwards excursion of their blood glucose levels ranging up to 300 mg/dL and then dropping rapidly back into the normal range as the insulin takes effect. The nondiabetic subjects were expected to exhibit the standard glucose tolerance test profiles,
15 typically showing a peak in blood glucose levels between 150 mg/dL and 220 mg/dL from 45 minutes to 90 minutes after administering the glucose load, and then a rapid drop back to their normal baseline levels over the next hour or so.

(g) Following the administration of the glucose load or glucose load and insulin shot, the subjects had samples drawn, simultaneously, of ISF and finger prick whole blood at
20 five minute intervals for the next three to four hours. The sampling was terminated when the blood glucose levels in three successive samples indicate that the subject's glucose had stabilized.

Upon examination of the data, several features were apparent. In particular, for any specific batch of ELITE test strips, there exist a distinct shift in the output shown on the
25 glucometer in mg/dL glucose as compared to the level indicated on the blood. An elevated reading would be expected due to the lack of hematocrit in the ISF and to the normal differences in the electrolyte concentrations between the ISF and whole blood. Regardless of the underlying reasons for this shift in output, it was determined via comparison to a reference assay that the true ISF glucose levels are linearly related to the values produced by the ELITE
30 system, with the scaling coefficients constant for any specific batch of ELITE strips. Consequently, for the comparison of the ISF glucose levels versus the whole blood measurements, first order linear correction was applied to the ISF data as follows:
$$\text{ISF}_{\text{glucose}} = 0.606 * \text{ISF}_{\text{ELITE}} + 19.5.$$

This scaling of the output of the ELITE glucometer when used to measure ISF
35 glucose levels, allows one to examine, over the entire data set, the error terms associated with using ISF to estimate blood glucose levels. Of course, even with no linear scaling whatsoever, the correlations between the ISF glucose values and the blood glucose levels are the same as the scaled version.

Based on the majority of the published body of literature on the subject of ISF
40 glucose as well as preliminary data, it was originally expected that a 15 to 20 minute lag between the ISF glucose levels and the those presented in the whole blood from a finger stick

5 would be observed. This is not what the data showed when analyzed. Specifically, when each individual's data set is analyzed to determine the time shift required to achieve the maximum correlation between the ISF glucose levels and the blood glucose levels it was discovered that the worst case time lag for this set of subjects was only 13 minutes and the average time lag was only 6.2 minutes, with several subjects showing a temporal tracking that was almost
10 instantaneous (about 1 minute).

Based on the minimal amount of lag observed in this data set, the graph shown in FIG. 25 presents all ten of the glucose load tests, concatenated one after another on an extended time scale. The data are presented with no time shifting whatsoever, showing the high level of tracking between the ISF and blood glucose levels the entire clinical data set
15 being dealt with in exactly the same manner. If the entire data set is shifted as a whole to find the best temporal tracking estimate, the correlation between the ISF and blood glucose levels peaks with a delay of two (2) minutes at an r value of $r=0.97$. This is only a trivial improvement from the unshifted correlation of $r=0.964$. Therefore, for the remainder of the analysis the ISF values are treated with no time shift imposed on them. That is, each set of
20 blood and ISF glucose levels is dealt with as simultaneously collected data pairs.

After the unshifted Elite ISF readings had been scaled to reflect the proportional glucose present in the ISF, it was possible to examine the error associated with these data. The simplest method for this is to assume that the average of the two ELITE finger-stick blood glucose readings is in fact the absolutely correct value, and then to merely compare the scaled
25 ISF values to these mean blood glucose values. These data are as follows: Standard Deviation Blood-ISF, 13.4 mg/dL; Coefficient of Variance of ISF, 9.7%; Standard Deviation of the Two Elites, 8.3 mg/dL; and Coefficient of Variance of Blood (Miles), 6%.

As these data show, the blood based measurement already contains an error term. Indeed, the manufacturer's published performance data indicates that the ELITE system has a nominal
30 Coefficient of Variance (CV) of between 5% and 7%, depending on the glucose levels and the amount of hematocrit in the blood.

An additional look at the difference term between the ISF glucose and the blood glucose is shown in the form of a scatter plot in FIG. 26. In this figure, the upper and lower bounds of the 90% confidence interval are also displayed for reference. It is interesting to note that with
35 only two exceptions, all of the data in the range of blood glucose levels below 100 mg/dL fall within these 90% confidence interval error bars. This is important as the consequences of missing a trend towards hypoglycemia would be very significant to the diabetic user. That is, it would be much better to under-predict glucose levels in the 40 to 120 mg/dL than to over predict them.

Essentially, if one assumes that the basic assay error when the ELITE system is used on
40 ISF is comparable to the assay error associated with the ELITE's use on whole blood, then the Deviation of the ISF glucose from the blood glucose can be described as: